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Thalamic glutamate levels as a predictor of cortical response during executive functioning in subjects at high risk for psychosis

Fusar-Poli, P ; Stone, J M ; Broome, M R ; Valli, I ; Mechelli, A ; McLean, M A ; Lythgoe, D J ; O’Gorman, R L ; Barker, G J ; McGuire, P K

Abstract: CONTEXT: Alterations in glutamatergic neurotransmission and cerebral cortical dysfunction are thought to be central to the pathophysiology of psychosis, but the relationship between these 2 factors is unclear. OBJECTIVE: To investigate the relationship between brain glutamate levels and cortical response during executive functioning in people at high risk for psychosis (ie, with an at-risk mental state [ARMS]). DESIGN: Subjects were studied using functional magnetic resonance imaging while they performed a verbal fluency task, and proton magnetic resonance spectroscopy was used to measure their brain regional glutamate levels. SETTING: Maudsley Hospital, London, England. Patients and Other PARTICIPANTS: A total of 41 subjects: 24 subjects with an ARMS and 17 healthy volunteers (controls). MAIN OUTCOME MEASURES: Regional brain activation (blood oxygen level-dependent response); levels of glutamate in the anterior cingulate, left thalamus, and left hippocampus; and psychopathology ratings at the time of scanning. RESULTS: During the verbal fluency task, subjects with an ARMS showed greater activation than did controls in the middle frontal gyrus bilaterally. Thalamic glutamate levels were lower in the ARMS group than in control group. Within the ARMS group, thalamic glutamate levels were negatively associated with activation in the right dorsolateral prefrontal and left orbitofrontal cortex, but positively associated with activation in the right hippocampus and in the temporal cortex bilaterally. There was also a significant group difference in the relationship between cortical activation and thalamic glutamate levels, with the control group showing correlations in the opposite direction to those in the ARMS group in the prefrontal cortex and in the right hippocampus and superior temporal gyrus. CONCLUSIONS: Altered prefrontal, hippocampal, and temporal function in people with an ARMS is related to a reduction in thalamic glutamate levels, and this relationship is different from that in healthy controls.

DOI: <https://doi.org/10.1001/archgenpsychiatry.2011.46>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-55999>

Journal Article

Accepted Version

Originally published at:

Fusar-Poli, P; Stone, J M; Broome, M R; Valli, I; Mechelli, A; McLean, M A; Lythgoe, D J; O’Gorman, R L; Barker, G J; McGuire, P K (2011). Thalamic glutamate levels as a predictor of cortical response during executive functioning in subjects at high risk for psychosis. Archives of General Psychiatry, 68(9):881-990. DOI: <https://doi.org/10.1001/archgenpsychiatry.2011.46>

THALAMIC GLUTAMATE LEVELS PREDICT CORTICAL RESPONSE DURING EXECUTIVE FUNCTIONING IN SUBJECTS AT HIGH RISK FOR PSYCHOSIS

Authorline

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ABSTRACT

Context: Alterations in glutamatergic neurotransmission and cerebral cortical dysfunction are thought to be central to the pathophysiology of psychosis, but the relationship between these two factors is unclear.

Objective: To investigate the relationship between brain glutamate and cortical response during executive functioning in people at high risk for psychosis (with an “at risk mental state”; ARMS).

Design: Subjects were studied using fMRI while performing a verbal fluency task, and with 1-H Magnetic Resonance Spectroscopy (1H-MRS) to measure brain regional glutamate levels.

Patients and other participants: Forty-one subjects participated in the study: twenty-four ARMS subjects and seventeen healthy volunteers.

Main outcome measures: Regional brain activation (BOLD response), levels of glutamate in the anterior cingulate, left thalamus and left hippocampus, and psychopathology ratings at the time of scanning.

Results: During the verbal fluency task, ARMS subjects showed greater activation than controls in the middle frontal gyrus bilaterally. Thalamic glutamate levels were lower in the ARMS group than in controls. Within the ARMS sample, activation in the right dorsolateral prefrontal and left orbitofrontal cortex were negatively associated with thalamic glutamate levels, but positively associated with activation in the right hippocampus and in the temporal cortex bilaterally. There was also a significant group difference in the relationship between cortical activation and thalamic glutamate levels, with controls showing correlations in the opposite direction to those in the ARMS group in the prefrontal cortex, and in the right hippocampus and superior temporal gyrus.

Conclusions: Altered prefrontal, hippocampal and temporal function in people with an ARMS is related to a reduction in thalamic glutamate levels, and this relationship is different from that in healthy controls.

INTRODUCTION

Cognitive deficits are a robust feature of schizophrenia, and a major contributor to the substantial functional disability associated with the disorder. Compared to controls, patients with schizophrenia show impaired behavioural performance on tasks that engage executive functions¹, such as verbal fluency paradigms, and neuroimaging studies have shown that they differentially engage the prefrontal areas that normally mediate these processes during such tasks²⁻⁵. Recent studies have reported that qualitatively similar impairments in behavioural performance and alterations in prefrontal activation are also evident in subjects who have prodromal symptoms of psychosis, but are not frankly psychotic⁶⁻⁸. The prodromal symptoms of psychosis can be identified using standardized, structured assessments, based on a combination of clinical criteria: the presence of attenuated psychotic symptoms, or a family history of psychosis in connection with deterioration in social functioning, or a self-limiting psychotic episode which lasted less than one week⁹. Individuals meeting these criteria have an “At Risk Mental State” (ARMS) which is associated with a high risk of developing a psychotic disorder within two years¹⁰.

Functional magnetic resonance imaging (fMRI) studies of verbal fluency in the ARMS indicate that the level of activation in prefrontal cortex is intermediate between that in psychotic patients and that in controls¹¹, independent of differences in task performance or potential effects of antipsychotic medication. The relationship between prefrontal cortical dysfunction in this context and the neurochemical changes associated with psychosis is unclear. Over the past two decades there has been growing interest in the role of glutamate, the main excitatory neurotransmitter in the brain, in the pathophysiology of psychosis. Experimental administration of ketamine, an uncompetitive N-Methyl-D-Aspartate receptor (NMDA) antagonist, in healthy volunteers can produce impairments on tasks of executive function^{12, 13} and alterations in cortical activation during verbal fluency that are comparable to those seen in schizophrenia¹⁴. Animal models of psychosis suggest that blockade of NMDA receptors can lead to increased cortical glutamate release^{15, 16} and the loss of cortical neurons¹⁷. These effects appear to be driven by NMDA receptor blockade in the thalamus rather a direct effect on NMDA receptors in the cortex^{16, 18}. Proton magnetic resonance spectroscopy (1H-MRS) permits direct measurement of glutamate and glutamine (a marker of glutamate release) in living

human subjects. Recent 1H-MRS studies have shown an increase in anterior cingulate levels of glutamine, following the acute administration of ketamine ¹⁹, closely resembling the previously reported findings in animals ^{15, 16}. Abnormal anterior cingulate glutamine levels have also been reported in patients undergoing the first episode of schizophrenia ²⁰. We recently found altered anterior cingulate glutamine levels in subjects with an ARMS, but the most striking finding in this group was a reduction in thalamic glutamate levels, which was associated with reductions in prefrontal, temporal and hippocampal grey matter volume in the same individuals²¹.

The aim of the present study was to examine the relationship between the cortical response during a task of executive functioning and central glutamate levels in subjects with an ARMS. We selected this group because it is thought that glutamate dysfunction may be particularly important in the early stages of psychosis ^{20, 22, 23}, and because individuals with an ARMS are usually treatment naïve, and antipsychotic medication may alter both cortical activation ²⁴ and glutamate activity ²⁵. We studied the same individuals using two different neuroimaging techniques: functional magnetic resonance imaging was used to assess cortical responses during a verbal fluency task, and 1H-MRS was used to measure regional glutamate levels. These techniques were applied to a sample of subjects with an ARMS and a demographically matching group of healthy volunteers. On the basis of previous studies, we predicted that, relative to controls, the ARMS group would show altered activation in prefrontal cortex ^{11, 26} while performing the verbal fluency task ²⁷, and reduced glutamate levels in the thalamus ²¹. We then tested our main hypothesis: that within the ARMS group, the severity of the alteration in cortical function would be related to the change in thalamic glutamate levels.

MATERIAL AND METHODS

Subjects

Individuals meeting criteria for the an At-Risk Mental State (ARMS) (n=24) were recruited from Outreach and Support in South London (OASIS) ²⁸. The diagnosis was based on assessment by two experienced clinicians, using the Comprehensive Assessment for the ARMS (CAARMS) ⁹, as well as a consensus meeting with the clinical team. All subjects were antipsychotic naïve at the time of the scanning. Two

subjects were receiving antidepressant medication. The subjects were representative of the local population of people presenting with an ARMS in terms of age, gender, ethnicity, and duration and intensity of symptoms²⁸. Subjects were excluded if there was a history of neurological disorder or they met DSM-IV criteria for a substance dependence or abuse disorder. Controls (n=17) were recruited from the same geographical area via advertisements in the local media. Control subjects had no history of psychiatric symptoms, substance dependence / abuse, medical illness or use of psychotropic medications and no family history of psychiatric illness. All subjects but one (an ARMS subject) were right-handed, as evaluated using the Lateral Preferences Inventory²⁹, and all were native speakers of English.

Clinical measures

Prior to scanning, all subjects were interviewed about their family and personal psychiatric history, current and past medication use. The severity of psychotic symptoms in the two groups was assessed at the time of scanning using the CAARMS⁹ and the Positive and Negative Symptom Scale (PANSS)³⁰. Consumption of illicit substances, alcohol, tobacco and coffee/tea was evaluated using a modified version of the Cannabis Experience Questionnaire (CEQ)³¹. Affective symptoms were assessed with the Hamilton Depression and Anxiety Scales (HAM-D and HAM-A)³². Premorbid intelligence was assessed using the National Adult Reading Test (NART)³³. Although individual differences in the personality dimensions of introversion/extroversion may affect brain function during cognitive activity³⁴ and glutamate levels³⁵ we did not measure these personality domains in the present study. The effect of group on demographic and clinical measures was tested using analyses of variance for parametric variables, and Mann-Whitney *U* tests were used to compare individuals with ARMS to controls for nonparametric variables after checking for equality of variance with the Levene test.

fMRI scanning

Image acquisition

For each participant, T2*-weighted gradient-echo single-shot echo-planar images were acquired on a 1.5 Tesla Signa (General Electric, Milwaukee) system at the Maudsley Hospital, London. Images comprised of 14 noncontiguous axial planes (7-mm thickness, slice skip: 1 mm) parallel to the anterior commissure-

posterior commissure line and were acquired with TE=40 msec, flip angle=70°, a matrix size of 64x64 pixels and a field of view of 200mm. Because the experimental paradigm required subjects to articulate a verbal response, we used a "clustered" acquisition sequence^{36, 37}. A clustered acquisition sequence capitalizes on the delay of the haemodynamic response, which reaches its peak about 3000–5000 msec after stimulus onset³⁸. A letter cue was presented for 750 msec and an overt verbal response could be made over a silent period of 2900 msec; an image was then acquired over 1100 msec resulting in a total repetition time (TR) of 4000 msec.

Verbal Fluency task (VF). Subjects were required to overtly articulate a word beginning with a visually presented letter. The stimuli, each subtending an angle of 5°, were presented in white on a black screen, viewed through a mirror. Cognitive load was modulated with 2 levels of task difficulty: 'easy' and 'hard' conditions using letters that differed with respect to the ease with which volunteers can usually generate words in response to them. The 'easy' condition involved the letters L, T, C, P, S; the 'hard' condition O, N, E, F, G³. Incorrect responses were defined as words that were proper names, repetitions or grammatical variations of the previous word, and 'pass' responses. Letters were presented in 28 second (s) blocks of 7 stimuli at 4 s intervals. The control condition of word repetition comprised 28 s blocks of 7 presentations of the word 'rest' at 4 s intervals, which subjects were required to read aloud. Five blocks of each condition (Hard/Easy/Repetition) were presented in random order. Verbal responses were recorded via a MRI compatible microphone using Cool Edit 2000 software (Syntrillium Software Corporation). To ensure that subjects heard their responses clearly, their speech was transmitted by a MRI compatible microphone, amplified by a computer sound card, and relayed back through an acoustic MRI sound system (Ward Ray, Hampton Court, UK) and noise insulated, stereo headphones at a volume of 91 ± 2 dB. The effect of group on response accuracy during scanning was tested using an ANOVA.

Analysis of fMRI data

Functional MRI data were analyzed using Statistical Parametric Mapping (SPM5; Wellcome Department of Cognitive Neurology, London, United Kingdom) running in MATLAB7.1. All volumes were realigned to the first volume, corrected for motion, mean adjusted by proportional scaling, normalized into standard stereotactic space (template provided by the Montreal Neurological Institute) and smoothed using a 6mm

full-width-at half-maximum Gaussian kernel. The time series were high pass filtered to eliminate low-frequency components (filter width 128 s). In the first level analysis the onset times (in seconds) for each trial was convolved with a canonical haemodynamic response function. Because no significant effect of cognitive load on regional activation was observed, each task condition (easy, hard) was then contrasted against the baseline condition (rest) in each subject. Because group differences in task performance could contribute to group differences in activation, the analysis was restricted to images associated with correct responses. To test the hypothesis that there were between-group differences, we performed a second level analysis comparing the activation during verbal fluency, independent of task demand (easy and hard word generation combined versus word repetition) between the two groups (controls and ARMS), using an ANOVA between-subjects test. As we were testing an *a-priori* hypothesis regarding group effects in the frontal lobe^{11, 39}, we used a frontal lobe mask generated by WFU Pickatlas (<http://www.fmri.wfubmc.edu/>)⁴⁰ for second-level group contrasts. The mask included frontal regions corresponding to Brodmann areas (BA) 4, 6, 8, 9, 46, 10, 11, 47, 45, 44, 32, and 24. The whole brain voxel-wise threshold was set at $p < .05$ [FWE-corrected]²⁷.

1H-MRS scanning

Volumetric MRI acquisition

The fMRI and MRS scans in each subject were performed as close together as was practically possible. In many subjects both scans were performed in the same week, but restrictions on the availability of scanning slots meant that this was not always possible. All MRS scanning took place on a General Electric (Milwaukee, USA) 3T MR system at the Centre for Neuroimaging Sciences. After positioning the subject in the scanner with earplugs and a foam rest under their knees, they were scanned with an initial three-plane localiser scan. This was used to measure the interhemispheric angle and the AC-PC line (the line passing through the upper part of the anterior commissure and the lower part of the posterior commissure). This was followed by an axial 2D T₂ weighted Fast Spin Echo scan and an axial fast FLAIR (Fluid Attenuated Inversion Recovery) scan, both prescribed parallel to the AC-PC line, which together were used for visual assessment to exclude any gross structural abnormality. These were followed by a whole brain 3D coronal

IR-SPGR (inversion recovery prepared spoiled gradient echo) scan, prescribed from the midline sagittal localiser images, giving isotropic 1.1mm voxel size (TE = 2.82ms; TR = 6.96ms; TI = 450ms; excitation flip angle = 20°) in a scan time of approximately 6 minutes. The IR-SPGR scans were used for localisation of the spectroscopy ROIs, and were subsequently segmented into grey matter, white matter and CSF using SPM2 to allow correction of the spectroscopy results for partial volume CSF contamination.

1H-MRS protocol

MRS spectra (PRESS - Point RESolved Spectroscopy - TE=30ms, TR=3000ms, 96 averages) were acquired in the anterior cingulate, left hippocampus and left thalamus. An automated shimming and water suppression method was used⁴¹, and the auto-prescan was performed twice prior to each scan. The centre of the 20mm x 20mm x 20mm anterior cingulate Region of Interest (ROI) was placed 13mm above the anterior section of the Genu of Corpus Callosum at 90° to the AC-PC line (Figure 1). A 20mm x 20mm x 15mm (right-left, anterior-posterior, superior-inferior) left hippocampal voxel was prescribed from the coronal IR-SPGR (Figure 1). A 15mm x 20mm x 20mm (right-left, anterior-posterior, superior-inferior) left thalamic voxel was defined at the point in the coronal slices where the thalamus was widest, using sagittal and coronal views to ensure that the voxel was clear of CSF contamination (Figure 1). For each metabolite spectrum, 16 unsuppressed water reference lines were also acquired as part of the standard PROBE acquisition (GE medical systems). We aimed for a maximum linewidth (FWHM) of the water peak at prescan of 7Hz for the anterior cingulate voxel, 10 Hz for the thalamus, and 11 Hz for the hippocampus. After the subject left the scanner, each scanning session concluded with the collection of a PRESS spectrum from a phantom containing standard concentrations of brain metabolites to provide calibration data for the LCModel program.

*** FIGURE 1 ABOUT HERE ***

1H-MRS quantification

All spectra were analysed using LCModel version 6.1-4F⁴². The raw spectral data were read into LCMgui, the graphical user interface for LCModel, which automatically combined the data from the eight-channel coil with a weighted coherent average over the eight receive channels using the intensity of the first point of the Free Induction Decay of the unsuppressed water reference from each coil. A standard basis set of 16

metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, taurine), included as part of LCModel and acquired with the same field strength (3-T), localization sequence (PRESS), and echo time (30 msec) as our study was used. Model metabolites and concentrations employed in the basis set are fully detailed in the LCModel manual (<http://s-provencher.com/pages/lcm-manual.shtml>). The brain tissue content in the region corresponding to each ROI was determined through matching its location from the 1H-MRS file headers with the same region in the segmented IR-SPGR images. Water-scaled glutamate values were divided by the brain tissue (gray plus white matter) content of the voxel in each subject (CSF-corrected). Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds of more than 20% reported by LCModel) were excluded from further analysis.

Integration of 1H-MRS and fMRI data

The relationship between the BOLD response during the VF task and glutamate levels was investigated by entering the glutamate measures as covariates in the independent sample t-test analysis of fMRI data. Glutamate-BOLD response interactions were assessed using whole brain regressions, conducted separately in ARMS and controls. In a second step, we explored the group by BOLD by glutamate interaction by modelling these factors in a different SPM design matrix. For all these contrasts whole brain voxel-wise threshold was set at $p < .05$ FWE-corrected. Post-hoc analyses were performed to evaluate the strength of these associations by extracting the beta values and testing them in a regression model in SPSS. Cook's distance test was used to assess the effect of potential outliers on the above correlations.

RESULTS

Clinical characteristics of the sample

Control and ARMS individuals did not differ in age (ARMS mean=26.6 [5] years, controls mean [SD]=25.5 [3.6] years, $t=1.490$, $p=0.146$), estimated premorbid IQ (control mean [SD] =102.6 [9.2], ARMS mean [SD]=101.7 [12.3]; $t=1.774$, $p=0.085$) or gender (ARMS females $n=1$, control females $n=7$, $X^2=1.502$, $p=0.220$), but controls had a significantly higher level of education than ARMS individuals ($X^2=0.049$).

There were no significant group differences in substance or alcohol use ($p > 0.05$). ARMS subjects had higher levels of prodromal, psychotic, anxiety and depressive symptoms than controls, as measured using the CAARMS (thought disorders severity, $t = 7.789$, $p < 0.001$; perceptual disorders severity, $t = 3.377$, $p = 0.002$; speech disorders severity, $t = 2.194$, $p = 0.035$), PANSS (ARMS mean positive [SD] = 12.67 [3.67], control mean positive [SD] = 7.35 [1.01], $t = 4.667$, $p < 0.001$; ARMS mean negative [SD] = 8.95 [2.71], control mean negative [SD] = 7.07 [0.26], $t = 2.583$, $p = 0.014$; ARMS mean general [SD] = 21.5 [4.2], control mean general [SD] = 16.5 [0.77], $t = 4.399$, $p < 0.001$), HAM-A (ARMS mean [SD] = 11.29 [3.44], control mean [SD] = 1.70 [0.75], $t = 3.308$, $p = 0.002$) and HAM-D (ARMS mean [SD] = 8.83 [3.14], control mean [SD] = 0.71 [0.32], $t = 3.686$, $p = 0.001$), respectively. No significant correlations between glutamate levels or regional activation and psychotic, anxious or depressive symptoms were elicited. We did not try to subdivide the ARMS sample according to whether they developed psychosis subsequent to scanning, as the sample is still undergoing clinical follow up. At least two years follow up is needed to determine which individuals will become psychotic⁴³.

MRS results

1H-MRS spectra quality were good in left thalamus and in anterior cingulate, with a mean (\pm SD) signal-to-noise ratio reported by LCModel of 19 (\pm 4) and 19 (\pm 6) respectively, and of reasonable quality in left hippocampus with a mean (\pm SD) signal-to-noise ratio of 12 (3). Linewidths reported by LCModel followed a similar pattern with mean (\pm SD) of 5.3 (\pm 1.8) Hz in anterior cingulate, 6.6 (\pm 1.4) Hz in left thalamus, and 8.9 (\pm 3.1) Hz in left hippocampus. There were no significant differences in spectral quality between control and ARMS subjects. ARMS subjects had lower glutamate levels ($t = 2.727$, $p = 0.009$) in left thalamus than controls. There was also a trend towards lower glutamate levels in the ARMS group than in controls in the left hippocampus ($t = 1.937$, $p = 0.063$). There were no significant group differences in glutamate levels in the anterior cingulate gyrus ($p > 0.05$).

Verbal fluency task

Performance

There was no significant difference in the accuracy of responses during the verbal fluency task between the ARMS and control groups ($p=0.109$).

Regional activation

Main effect of task

Across all subjects, word generation relative to word repetition was associated with activation in several regions of the cerebral cortex, with a significant lateralization effect on the left. Brain areas activated by the task included the inferior frontal gyrus bilaterally, and the left insula, superior frontal gyrus, and anterior cingulate gyrus ($p<0.05$). There was also subcortical activation in the left putamen and globus pallidus (Table 1s and Figure 1s; see supplementary materials)($p<0.05$).

Main effect of group

The ARMS group showed greater activation than controls in the middle frontal gyrus bilaterally (Table 1s and Figure 2)($p<0.05$). Conversely, no brain areas showed greater activation in the control group than the ARMS group.

FIGURE 2 ABOUT HERE

Correlation between 1H-MRS and fMRI

Thalamus

Within-group correlations

No significant correlations between cortical activation during verbal fluency and thalamic glutamate levels were observed in healthy controls. However, in the ARMS sample, thalamic glutamate levels were negatively associated with activation in the right dorsolateral prefrontal cortex (right middle frontal gyrus; $R=0.784$, $R^2=0.614$, $p<0.001$) and the left orbitofrontal cortex (left middle frontal gyrus; $R=0.602$, $R^2=0.362$, $p=0.03$), but positively associated with activation in the temporal cortex bilaterally (right superior

temporal gyrus; $R=0.801$, $R^2=0.642$, $p<0.001$; left middle temporal gyrus, $R=0.465$, $R^2=0.216$, $p=0.034$) and in the right hippocampus ($R=0.605$, $R^2=0.366$, $p=0.04$), but (Figure 3, Table I).

Differences between groups

There were significant interactions between these findings and group ($p<0.05$). In the prefrontal cortex bilaterally (left middle frontal and right superior frontal gyri), thalamic glutamate levels were negatively associated with activation in the ARMS, but positively associated with activation in controls (Figure 4, Table I). Conversely, thalamic glutamate was positively correlated with brain activation in the right hippocampus and superior temporal gyrus in the ARMS group, but negatively correlated in the control group.

*** FIGURE 3 ABOUT HERE ***

*** TABLE I ABOUT HERE ***

*** FIGURE 4 ABOUT HERE ***

Hippocampus and Anterior cingulate

No significant correlations between cortical response during verbal fluency and glutamate levels in either the hippocampus or anterior cingulate cortex were observed in ARMS or in healthy controls, and there were no significant group by glutamate by cortical activation interactions.

DISCUSSION

To our knowledge this is the first study to explore the relationship between glutamate levels and cortical activation in relation to psychosis in human subjects. Consistent with data from previous investigations^{21, 44}, thalamic glutamate level was lower in the ARMS than in the controls, and ARMS subjects showed an increased frontal activation during the verbal fluency task²⁷. Within the ARMS sample, the glutamate level in the thalamus was positively associated with activation in the temporal cortex and hippocampus, but negatively associated with activation in the prefrontal cortex. Moreover, significant interactions between these findings and group were detected in the prefrontal and temporal cortex and in the hippocampus, indicating that the relationship between activation and glutamate levels was different in ARMS subjects and controls.

In keeping with previous studies in healthy controls³⁹ and in patients with schizophrenia⁴⁵, our verbal fluency task preferentially activated the left prefrontal cortex, and this region was differentially activated in ARMS subjects and controls, with a greater BOLD response bilaterally in the former group. These differences in activation were evident in medication-naïve subjects²⁴, in the context of similar levels of task performance. Moreover, the analysis was restricted to images associated with correct responses. The findings may thus reflect a true difference at the neurophysiological level, as opposed to a non-specific effect of differential task performance. Greater engagement of prefrontal cortex in clinical subjects has been interpreted as a manifestation of inefficient prefrontal processing⁴⁶, and may underlie the behavioural impairment on executive functions that has consistently been observed in neuropsychological studies in ARMS subjects^{47, 48}. Our fMRI findings are consistent with evidence that grey matter volume in prefrontal regions is also abnormal in the ARMS⁴⁹⁻⁵¹, as well as in first episode psychosis⁵² and in chronic schizophrenia⁵³.

The finding of reduced glutamate levels in the thalamus in ARMS subjects replicates a previous finding in a larger overlapping sample²¹, which contained some of the present ARMS group. It is not attributable to an effect of antipsychotic medication, as most of the ARMS subjects were medication naïve, nor to an effect of chronic illness, as none of these subjects had ever been frankly psychotic. Our main finding was that the relationship between thalamic glutamate levels and neuronal activation differed between the ARMS and control groups, particularly in the prefrontal and lateral temporal cortices and in the hippocampus. In the lateral temporal and hippocampal regions, the different relationship between thalamic glutamate levels and neuronal activation could be detected in the absence of different brain activation suggesting that, in these regions, alterations in glutamate levels may occur independently of cortical dysfunction.

These findings taken together are consistent with evidence that administration of ketamine, an uncompetitive NMDA receptor antagonist, modulates activation during verbal fluency and resting state activity in these regions in healthy volunteers^{14, 54, 55}. Furthermore, we have previously shown that the level of thalamic glutamate in the ARMS is also correlated with gray matter volume in the prefrontal, temporal polar and medial temporal cortex²¹. A study that combined 1H-MRS and MRI in schizophrenia found that there was a longitudinal reduction in thalamic glutamine (a precursor of glutamate) levels that was associated with a

progressive reduction in temporal gray matter volume⁵⁶. The prefrontal, temporal polar and hippocampal cortex have been widely implicated in the pathogenesis of psychotic disorders⁵⁰, and the present findings suggest that perturbed subcortical glutamate levels may contribute to alterations in the function of these areas. The localisation of the correlations with glutamate to the prefrontal, temporal and hippocampal cortex is also consistent with post mortem evidence that these regions are sites of glutamatergic abnormalities in schizophrenia⁵⁷.

The thalamus plays a key role in cognitive functioning, and prefrontal-thalamic interactions mediate the state of consciousness, the sleep-wake cycle, and executive functions⁵⁸, including those engaged during verbal fluency tasks⁵⁹. A recent study employing simultaneous EEG recordings from deep brain structures and the scalp showed that the human thalamus systematically reacts to language analysis in coordination with cortical regions⁶⁰. Neuropathological and in vivo imaging studies in schizophrenia have identified several structural and functional abnormalities in the thalamus⁶¹, and these changes have been related to cognitive impairments⁶² and symptomatology in psychosis⁶³. It is thus plausible that abnormalities in thalamic neurotransmission could contribute to the development of psychosis.

The relationship between thalamic glutamate levels and cortical activation during verbal fluency in the ARMS sample was significantly different to that in controls. In the ARMS subjects, thalamic glutamate levels were negatively associated with the prefrontal cortical response, whereas the correlation was positive in controls, although it was not statistically significant. The negative correlation between frontal activation and thalamic glutamate levels within the ARMS group suggests that the greater the neurophysiological abnormality in the frontal cortex, the greater the neurochemical abnormality in the thalamus. Similarly, the positive correlations with activation in the right hippocampus and superior temporal gyrus in the ARMS were absent in controls, who showed a tendency for correlations in a negative direction. Interestingly, there were no interactions in those areas with normal glutamate values (hippocampus and anterior cingulate). This is consistent with the notion that the group differences in the correlations between activation and thalamic glutamate may have been a function of the group difference in glutamate values in the thalamus.

The correlation coefficients in the ARMS group were strong (ranging from 0.46 to 0.8), suggesting that there is a close relationship between the alterations in glutamate levels and in cortical function in people with an enhanced risk of psychosis. The opposite direction of the associations between glutamate and activation in the prefrontal and temporal cortices may reflect the opposite direction of the BOLD responses in the respective regions during verbal fluency tasks, with activation in prefrontal areas, but deactivation in temporal areas⁶⁴. Nevertheless, regardless of the directions of the correlations, there were significant differences in the relationship between glutamate levels and cortical response in ARMS subjects compared to controls.

The healthy brain may compensate for the potential effects of inter-subject variations in thalamic glutamate levels on cortical function, or vice versa. However, in the ARMS, the regulation of thalamic and / or cortical function may be compromised, such that variations in glutamate levels can directly affect cortical function. However, because it is not possible to determine the direction of causality from our data, it might equally reflect prefrontal dysfunction resulting in altered top-down influences on thalamic glutamate levels. While we observed a significant correlation between glutamate levels and cortical activation, there was no evidence of a correlation between glutamate levels and performance on the verbal fluency task ($p > 0.05$). Task performance can be seen as an indirect measure of the underlying cortical physiology, and may be influenced by a range of additional factors. It is thus not surprising that we were able to detect a relationship between glutamate levels and cortical activation, but not with task performance. The latter might have been evident if there had been significant group differences in task performance, and if the study had been powered to detect differences at the behavioural, as opposed to the physiological level.

About a third of subjects with an ARMS will subsequently develop psychosis, some will continue to experience prodromal symptoms but not become psychotic, while others will recover to the extent that they no longer meet criteria for the ARMS⁶⁵. Given the cross-sectional nature of the present study, it remains to be determined whether the observed glutamatergic and prefrontal alterations represent “state” risk factors, or are true “trait” vulnerability markers specifically linked to the later onset of psychosis.^{44, 66}. This issue will be addressed by follow up of the present sample to determine their long term clinical outcome. This entails

clinical monitoring for at least 24 months post scanning (as most transitions to psychosis occur during this period⁴³), and at present most of the sample are still undergoing follow up. Although we focused on glutamatergic abnormalities in the thalamus, pathways linking the thalamus and the cerebral cortex also involve GABAergic and dopaminergic neurotransmission⁶³, and GABA and dopamine may also play a crucial role in the pathophysiology of psychosis^{67 27}.

CONCLUSION

In subjects with prodromal signs of psychosis, altered prefrontal, temporal and hippocampal responses during verbal fluency were related to a reduction in thalamic glutamate levels. This relationship was not evident in controls, and may underlie the increased vulnerability to psychosis that is evident in the former group.

REFERENCES:

1. Reichenberg A, Harvey PD. Neuropsychological impairments in schizophrenia: Integration of performance-based and brain imaging findings. *Psychol Bull.* Sep 2007;133(5):833-858.
2. Curtis VA, Bullmore ET, Brammer MJ, et al. Attenuated frontal activation during a verbal fluency task in patients with schizophrenia. *Am J Psychiatry.* Aug 1998;155(8):1056-1063.
3. Fu CH, Morgan K, Suckling J, et al. A functional magnetic resonance imaging study of overt letter verbal fluency using a clustered acquisition sequence: greater anterior cingulate activation with increased task demand. *Neuroimage.* Oct 2002;17(2):871-879.
4. Boksman K, Theberge J, Williamson P, et al. A 4.0-T fMRI study of brain connectivity during word fluency in first-episode schizophrenia. *Schizophr Res.* Jun 15 2005;75(2-3):247-263.
5. Schaufelberger M, Senhorini MC, Barreiros MA, et al. Frontal and anterior cingulate activation during overt verbal fluency in patients with first episode psychosis. *Rev Bras Psiquiatr.* Sep 2005;27(3):228-232.
6. Simon AE, Cattapan-Ludewig K, Zmilacher S, et al. Cognitive functioning in the schizophrenia prodrome. *Schizophr Bull.* May 2007;33(3):761-771.
7. Lencz T, Smith CW, McLaughlin D, et al. Generalized and specific neurocognitive deficits in prodromal schizophrenia. *Biol Psychiatry.* May 1 2006;59(9):863-871.
8. Pukrop R, Ruhrmann S, Schultze-Lutter F, Bechdolf A, Brockhaus-Dumke A, Klosterkötter J. Neurocognitive indicators for a conversion to psychosis: comparison of patients in a potentially initial prodromal state who did or did not convert to a psychosis. *Schizophr Res.* May 2007;92(1-3):116-125.
9. Yung AR, Yuen HP, McGorry PD, et al. Mapping the onset of psychosis: the Comprehensive Assessment of At-Risk Mental States. *Aust N Z J Psychiatry.* Nov-Dec 2005;39(11-12):964-971.
10. Cannon TD, Cornblatt B, McGorry P. The empirical status of the ultra high-risk (prodromal) research paradigm. *Schizophr Bull.* May 2007;33(3):661-664.
11. Fusar-Poli P, Perez J, Broome MR, et al. Neurofunctional correlates of liability to psychosis: a systematic review and meta-analysis. *Neur Biob Rev.* 2007;31(4):465-484.
12. Krystal JH, Karper LP, Seibyl JP, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry.* Mar 1994;51(3):199-214.
13. Morgan CJ, Curran HV. Acute and chronic effects of ketamine upon human memory: a review. *Psychopharmacology (Berl).* Nov 2006;188(4):408-424.
14. Fu CH, Abel KM, Allin MP, et al. Effects of ketamine on prefrontal and striatal regions in an overt verbal fluency task: a functional magnetic resonance imaging study. *Psychopharmacology (Berl).* Nov 2005;183(1):92-102.
15. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci.* Apr 15 1997;17(8):2921-2927.
16. Lorrain DS, Baccei CS, Bristow LJ, Anderson JJ, Varney MA. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. *Neuroscience.* 2003;117(3):697-706.
17. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry.* Dec 1995;52(12):998-1007.
18. Sharp FR, Tomitaka M, Bernaudin M, Tomitaka S. Psychosis: pathological activation of limbic thalamocortical circuits by psychomimetics and schizophrenia? *Trends Neurosci.* Jun 2001;24(6):330-334.
19. Rowland LM, Bustillo JR, Mullins PG, et al. Effects of ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. *Am J Psychiatry.* Feb 2005;162(2):394-396.
20. Theberge J, Al-Semaan Y, Williamson PC, et al. Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. *Am J Psychiatry.* Dec 2003;160(12):2231-2233.

21. Stone J, Day F, Tsagaraki H, et al. Glutamate dysfunction in people with prodromal symptoms of psychosis: relationship to gray matter volume. *Mol Psychiatry*. 2009;submitted.
22. Tibbo P, Hanstock C, Valiakalayil A, Allen P. 3-T proton MRS investigation of glutamate and glutamine in adolescents at high genetic risk for schizophrenia. *Am J Psychiatry*. Jun 2004;161(6):1116-1118.
23. Theberge J, Bartha R, Drost DJ, et al. Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. *Am J Psychiatry*. Nov 2002;159(11):1944-1946.
24. Fusar-Poli P, Broome MR, Matthiasson P, Williams SC, Brammer M, McGuire PK. Effects of acute antipsychotic treatment on brain activation in first episode psychosis: an fMRI study. *Eur Neuropsychopharmacol*. May-Jun 2007;17(6-7):492-500.
25. Marek GJ, Behl B, Besspalov AY, Gross G, Lee Y, Schoemaker H. Glutamatergic (N-methyl-D-aspartate receptor) hypofrontality in schizophrenia: too little juice or a miswired brain? *Mol Pharmacol*. Mar 2010;77(3):317-326.
26. Broome MR, Matthiasson P, Fusar-Poli P, et al. Neural correlates of executive function and working memory in the 'at-risk mental state'. *Br J Psychiatry*. 2009;194(1):25-33.
27. Fusar-Poli P, Howes OD, Allen P, et al. Abnormal prefrontal activation directly related to pre-synaptic striatal dopamine dysfunction in people at clinical high risk for psychosis. *Mol Psychiatry*. Dec 1 2009.
28. Broome M, Woolley J, Tabraham P, et al. What causes the onset of psychosis? *Schizophr Res*. 2005;79(1):23-34.
29. Coren S. Measurement of handedness via self-report: the relationship between brief and extended inventories. *Percept Mot Skills*. Jun 1993;76(3 Pt 1):1035-1042.
30. Kay SR. Positive-negative symptom assessment in schizophrenia: psychometric issues and scale comparison. *Psychiatr Q*. Fall 1990;61(3):163-178.
31. Barkus EJ, Stirling J, Hopkins RS, Lewis S. Cannabis-induced psychosis-like experiences are associated with high schizotypy. *Psychopathology*. 2006;39(4):175-178.
32. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry*. Feb 1960;23:56-62.
33. Nelson HE, Willison JR. *National Adult Reading Test (NART): Test Manual 2nd ed*. Windsor, England: NFER-Nelson 1991.
34. Kumari V, Ffytche D, Williams S, Gray J. Personality predicts brain responses to cognitive demands. *J Neurosci*. 2004;24(47):10636-10641.
35. Hoerst M, Weber-Fahr W, Tunc-Skarka N, et al. Correlation of glutamate levels in the anterior cingulate cortex with self-reported impulsivity in patients with borderline personality disorder and healthy controls. *Arch Gen Psychiatry*. Sep 2010;67(9):946-954.
36. Bullmore ET, Brammer MJ, Rabe-Hesketh S, et al. Methods for diagnosis and treatment of stimulus-correlated motion in generic brain activation studies using fMRI. *Hum Brain Mapp*. 1999;7(1):38-48.
37. Amaro E, Williams S, Shergill S, et al. Acoustic noise and functional magnetic resonance imaging: current strategies and future prospects. *J Magn Reson Imaging* 2002;16:497-510.
38. Glover GH. Deconvolution of impulse response in event-related BOLD fMRI. *Neuroimage*. 1999;9(4):416-429.
39. Costafreda SG, Fu CH, Lee L, Everitt B, Brammer MJ, David AS. A systematic review and quantitative appraisal of fMRI studies of verbal fluency: role of the left inferior frontal gyrus. *Hum Brain Mapp*. Oct 2006;27(10):799-810.
40. Maldjian J, Laurienti P, Kraft R, Burdette J. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage* 2003;19(3):1233-1239.
41. Webb PG, Sailasuta N, Kohler SJ, Raidy T, Moats RA, Hurd RE. Automated single-voxel proton MRS: technical development and multisite verification. *Magn Reson Med*. Apr 1994;31(4):365-373.
42. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*. Dec 1993;30(6):672-679.
43. Cannon TD, Cadenhead K, Cornblatt B, et al. Prediction of psychosis in youth at high clinical risk: a multisite longitudinal study in North America. *Arch Gen Psychiatry*. Jan 2008;65(1):28-37.

44. Stone JM, Fusar-Poli P. Abnormal thalamic glutamate and liability to psychosis: state or trait marker? *Schizophr Res*. Nov 2009;115(1):94-95.
45. Fu CH, Suckling J, Williams SC, Andrew CM, Vythelingum GN, McGuire PK. Effects of psychotic state and task demand on prefrontal function in schizophrenia: an fMRI study of overt verbal fluency. *Am J Psychiatry*. Mar 2005;162(3):485-494.
46. Tan HY, Callicott JH, Weinberger DR. Dysfunctional and compensatory prefrontal cortical systems, genes and the pathogenesis of schizophrenia. *Cereb Cortex*. Sep 2007;17 Suppl 1:i171-181.
47. Eastvold AD, Heaton RK, Cadenhead KS. Neurocognitive deficits in the (putative) prodrome and first episode of psychosis. *Schizophr Res*. Jul 2007;93(1-3):266-277.
48. Pukrop R, Schultze-Lutter F, Ruhrmann S, et al. Neurocognitive functioning in subjects at risk for a first episode of psychosis compared with first- and multiple-episode schizophrenia. *J Clin Exp Neuropsychol*. Nov 2006;28(8):1388-1407.
49. Borgwardt SJ, Riecher-Rossler A, Dazzan P, et al. Regional gray matter volume abnormalities in the at risk mental state. *Biol Psychiatry*. May 15 2007;61(10):1148-1156.
50. Pantelis C, Velakoulis D, McGorry PD, et al. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet*. Jan 25 2003;361(9354):281-288.
51. Meisenzahl EM, Koutsouleris N, Gaser C, et al. Structural brain alterations in subjects at high-risk of psychosis: a voxel-based morphometric study. *Schizophr Res*. Jul 2008;102(1-3):150-162.
52. Garcia-Marti G, Aguilar EJ, Lull JJ, et al. Schizophrenia with auditory hallucinations: a voxel-based morphometry study. *Prog Neuropsychopharmacol Biol Psychiatry*. Jan 1 2008;32(1):72-80.
53. Antonova E, Kumari V, Morris R, et al. The relationship of structural alterations to cognitive deficits in schizophrenia: a voxel-based morphometry study. *Biol Psychiatry*. Sep 15 2005;58(6):457-467.
54. Deakin JF, Lees J, McKie S, Hallak JE, Williams SR, Dursun SM. Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. *Arch Gen Psychiatry*. Feb 2008;65(2):154-164.
55. Lahti AC, Holcomb HH, Medoff DR, Tamminga CA. Ketamine activates psychosis and alters limbic blood flow in schizophrenia. *Neuroreport*. Apr 19 1995;6(6):869-872.
56. Theberge J, Williamson KE, Aoyama N, et al. Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. *Br J Psychiatry*. Oct 2007;191:325-334.
57. Deakin JF, Simpson MD. A two-process theory of schizophrenia: evidence from studies in post-mortem brain. *J Psychiatr Res*. Mar-Apr 1997;31(2):277-295.
58. Zikopoulos B, Barbas H. Parallel driving and modulatory pathways link the prefrontal cortex and thalamus. *PLoS ONE*. 2007;2(9):e848.
59. Marzinzik F, Wahl M, Schneider GH, Kupsch A, Curio G, Klostermann F. The human thalamus is crucially involved in executive control operations. *J Cogn Neurosci*. Oct 2008;20(10):1903-1914.
60. Wahl M, Marzinzik F, Friederici AD, et al. The human thalamus processes syntactic and semantic language violations. *Neuron*. Sep 11 2008;59(5):695-707.
61. Byne W, Hazlett EA, Buchsbaum MS, Kemether E. The thalamus and schizophrenia: current status of research. *Acta Neuropathol*. Apr 2009;117(4):347-368.
62. Habets P, Krabbendam L, Hofman P, et al. Cognitive Performance and Grey Matter Density in Psychosis: Functional Relevance of a Structural Endophenotype. *Neuropsychobiology*. Dec 16 2008;58(3-4):128-137.
63. Clinton SM, Meador-Woodruff JH. Thalamic dysfunction in schizophrenia: neurochemical, neuropathological, and in vivo imaging abnormalities. *Schizophr Res*. Aug 1 2004;69(2-3):237-253.
64. Schlosser R, Hutchinson M, Joseffer S, et al. Functional magnetic resonance imaging of human brain activity in a verbal fluency task. *Journal of Neurology Neurosurgery Psychiatry*. 1998;64:492-498.
65. Yung AR, Yuen HP, Berger G, et al. Declining transition rate in ultra high risk (prodromal) services: dilution or reduction of risk? *Schizophr Bull*. May 2007;33(3):673-681.
66. Blackwood D. P300, a state and a trait marker in schizophrenia. *Lancet*. Mar 4 2000;355(9206):771-772.

- 67.** Howes OD, Montgomery AJ, Asselin M, Murray R, Grasby P, McGuire P. Molecular imaging studies of the striatal dopaminergic system in psychosis and predictions for the prodromal phase of psychosis. *Br J Psychiatry*. 2007;s51:s13-s18.

Figure 1. Figure 1. Location of voxel placement for proton MRS acquisition: left hippocampus (upper left), anterior cingulate (upper right), right thalamus (lower left).

Figure 2. Between group differences in activation during the verbal fluency task. The ARMS group showed greater activation than controls (red clusters) in the right superior frontal gyrus and in the left middle frontal gyrus. The left side of the brain is shown on the left side of the figure.

FIGURE 3. Correlations between thalamic glutamate levels and brain activation in ARMS subjects during verbal fluency (positive correlations are shown in red and negative correlations in blue). Positive correlations were evident in the right superior temporal gyrus, hippocampus and in the left middle temporal gyrus. Negative correlations were evident in the middle frontal gyrus bilaterally.

Figure 4. Between group interactions between thalamic glutamate and cortical responses during verbal fluency. In the prefrontal cortex bilaterally (plots on the left of the figure), thalamic glutamate levels were negatively associated with activation in the ARMS group, but there was no significant correlation in controls. In the right hippocampus and superior temporal gyrus (right of the figure), the correlation in the ARMS sample was negative, while that in the controls was again non significant.